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Reference and troubleshooting guide

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Troubleshooting

Do you see a peak?

Yes

Low sensitivity?
Decreasing area response counts over the duration of a run?

- Compare problematic data with old data collected using the same acquisition method.
- Run an instrument tune with SCIEX-approved tuning solutions to see if the MS system still meets minimum sensitivity specifications.
- Run 30 replicate injections of a standard.

The system meets sensitivity specifications, but your data is still low.

- Verify that analytical standards are not degraded.
- Replace the analytical column with a new column.
- Replace with fresh mobile phase solutions.
- Test a different source if applicable.

The system is not meeting minimum sensitivity specifications or is showing signs of charging.

- Clean the ion source.
- Call service for an instrument cleaning.
- Test a different source if applicable.

Carryover?

Determine whether the carryover is from the analytical column or the injector port.

- Create an LC method where you double the mobile phase B ramp twice in the same method and inject 1 µL of air from an empty sample vial or well. Ensure that your acquisition method covers the entire duration of the LC method and the divert valve being used is programmed to send the sample to the MS.

The appearance of 2 sets of peaks in the run points to column carryover or mobile phase contamination.

- Make fresh mobile phases from clean glass bottles.
- Inject less material on the analytical column or dilute the sample more.
- Extend the high organic step for the analytical column in the original LC gradient to clean the column further.

If only 1 set of peaks appear, increase the needle rinsing volume step in the autosampler method or change the needle wash solution to a stronger wash for your analyte needs.

Bad peak shape?

Peak fronting is where peaks lose symmetry on the left most side of the peak and is usually caused by poor analyte retention on the analytical column.

- Decrease organic percentage in the sample solvent.
- Choose another analytical column chemistry with more retention.
- Load the sample onto the column using a high aqueous hold step in your LC gradient.

Peak tailing is where the peaks lose symmetry on the right most side of the peak and is an indicator that the column may be nearing the end of its lifespan.

- Replace either the analytical column or guard column (or both).

Check all fittings around the column to ensure no micro leak is causing the poor peak shape.

No

Equilibrate with your acquisition methods (LC and MS). Remove the PEEK sample line from the LC.

Do you see liquid dripping out of the line?

Yes

- Replace the line and tighten.
- Make sure the heater temperature of your acquisition method and nebulizer gas (GS1) is enough to fully desolvate your LC flow.
- If the front of the ion source glass window becomes foggy, increase the heater temperature of your method.
- If the method has already been validated and routinely used before with no issues, place a service call.
- Check that the MS acquisition method switches the divert valve to the MS at the correct time.

No

- Check that pump valves are closed.
- Check that there is enough mobile phase.
- Check that there are no air bubbles in the mobile phase lines. Purge if air bubbles can be seen.
- Equilibrate with the LC-MS/MS method. Check pump back pressure. There should be positive pressure.
- Check that there are no leaks. Run a lab wipe over the analytical columns and all fittings to check for small and slow leaks.

Still no peak?

Troubleshoot further to collect data for problem diagnosis.

OR

Contact technical support if you need help walking through the steps.

Are you using the Scheduled MRM Algorithm?

Yes

Uncheck the Scheduled MRM in the acquisition if more than 1 compound is missing. Run a medium to high solvent standard to verify the presence of compound.

Yes

Inject 3 consecutive samples with analytes.
Are there peaks at the wrong retention times?

Do peaks all coelute at once early in the gradient with no LC separation?

- Make new mobile phases using new glassware and purge pumps before LC-MS injections.
- Mobile phases A and B may be swapped.
- Analytes are not retained on column. Change to a retentive column chemistry for that specific analyte.
- Check LC pump output to check the performance of the pumps.

No

Deactivate the hardware profile, power cycle the MS and restart the computer. Wait 10 minutes and reactivate the hardware profile.

Still no peaks?

- Infuse your analyte via syringe infusion using a mix of 50:50 (v/v) of your mobile phases.
- Do you see your analyte precursor?
  - If yes: Choose new LC conditions. The compound is not eluting off the analytical column or being retained.
  - If no: Try a high concentration of analytical standard and/or check with published methods to verify precursor and fragment masses.

No

The system meets sensitivity specifications, but your data is still low.

- Verify that analytical standards are not degraded.
- Replace the analytical column with a new column.
- Replace with a fresh mobile phase.
- Test a different source if applicable.

Still no observable peak? Contact SCIEX technical support:
1-877-740-2129

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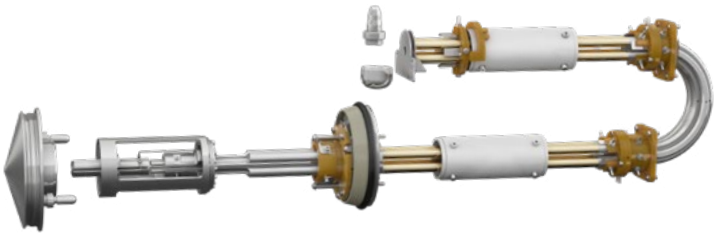
Triple quadrupole technology

Triple quadrupole mass spectrometers have long been the benchmark for quantitation due to their sensitivity and specificity. Combined with SCIEX QTRAP technology, a triple quadrupole mass spectrometer can also be used as a linear ion trap (LIT) for even more sensitivity and selectivity.



Triple quadrupole scans

Multiple reaction monitoring (MRM):

- MRM scans select specific precursor ions and fragment them to monitor specific productions.
- These scans are generally used for quantitation of a targeted list of compounds.

Precursor ion:

- With this scan, the third quadrupole (Q3) is set to a fixed mass and the first quadrupole (Q1) sweeps a mass range. It scans for the ion of a specific mass-to-charge ratio that is generating specific product ions.

Product ion:

- A product ion experiment searches for all of the products of a particular precursor fragmenting in the second quadrupole (Q2).
- It is generally used for compound optimization to determine product ions.

Neutral loss:

- This is a survey scan to monitor precursor ions that have a specific neutral loss.

Q1/Q3 full scan:

- This type of scan utilizes Q1 or Q3 as a mass filter to scan across a mass range or focus on certain ions with a specific mass window width.
- It is generally used to determine precursor masses for compound optimization.

QTRAP system scans

Enhanced MS (EMS):

- With an EMS scan, a specified precursor ion mass is trapped in the LIT before being released to the detector.

Enhanced resolution (ER):

- With an ER scan, ions within a 20 Da region are collected in the LIT for a specified fill time and scanned out slowly for enhanced resolution.

Enhanced product ion (EPI):

- An EPI scan is a triple quadrupole MS/MS scan where product ions are trapped in the LIT before hitting the detector.

Enhanced multiply charged (EMC):

- This is an MS scan in which multiply charged ions are detected within the specified range.

MS/MS/MS (MS^3)

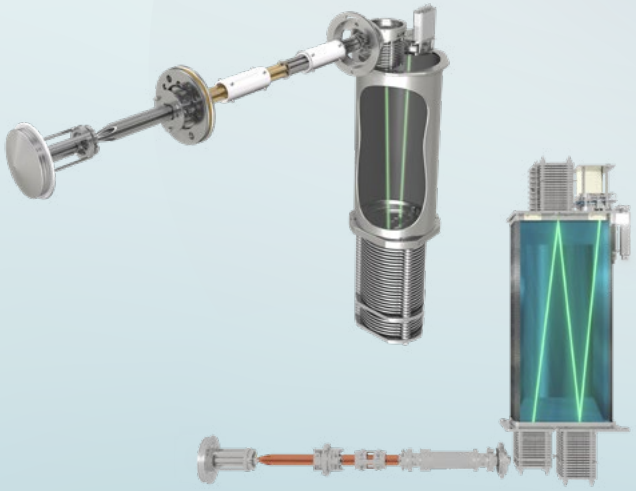
- With MS^3 scans, product ions of a specified m/z are isolated in the LIT and further fragmented. The resulting fragment ions are trapped into the LIT prior to being scanned out and detected.

Information dependent acquisition (IDA):

- An IDA experiment analyzes data as it is being acquired and changes experiment conditions according to the results of the analysis. It determines the masses on which to perform dependent scans.

Accurate mass technology

QTOF mass spectrometers combine quadrupole with time-of-flight technology, where ions travel through a flight tube to determine ion mass at high resolution, along with mass accuracy below 5 ppm. Fast scan speeds are necessary to maintain product ion resolution for enhanced spectral library matching.



QTOF scans

Information dependent acquisition (IDA):

- An IDA experiment analyzes data as it is being acquired and changes experimental conditions according to the results of the analysis. Analysis of the results determines the masses on which to perform dependent scans. The user has total control over the criteria that activates an IDA experiment and the parameters of the IDA experiment that are activated.

SWATH Acquisition:

- With this type of scan, the MS uses a specified Q1 isolation window and steps it across the entire m/z mass detection range, collecting full MS/MS spectra on every detectable analyte that passes through each Q1 window.
- With SWATH Acquisition, MS/MS data is never missed even if the peak signal intensity is low within a given mass window.

MRM^{HR}:

- With MRM^{HR} scans, MS/MS data is acquired from compounds with known masses and retention times with maximum selectivity. This acquisition can also be used to extract fragment masses with narrow widths (0.02 Da) from TOF MS/MS spectra.

Screening workflows

Targeted screening

- Compounds verified with analytical standards
- Highest confidence in compound ID

Suspect screening

- No analytical standards
- Spectral library matching with mass accuracy
- Analyte peak chosen based on molecular formula or target mass

Nontargeted screening

- No analytical standards
- Spectral library matching with mass accuracy
- Analyte peaks are all evaluated if minimum peak integration criteria is met

Call SCIEX technical support at 1-877-740-2129 or email customercare@sciex.com

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